

Art 34 amended

59
WHAT IS CLAIMED IS
-CLAIMS

1. A protein fragment having antimicrobial activity, wherein said protein fragment is selected from:

(i) a polypeptide having an amino acid sequence selected from:

residues 29 to 73 of SEQ ID NO: 1
residues 74 to 116 of SEQ ID NO: 1
residues 117 to 185 of SEQ ID NO: 1
residues 186 to 248 of SEQ ID NO: 1
residues 29 to 73 of SEQ ID NO: 3
residues 74 to 116 of SEQ ID NO: 3
residues 117 to 185 of SEQ ID NO: 3
residues 186 to 248 of SEQ ID NO: 3
residues 1 to 32 of SEQ ID NO: 5
residues 33 to 75 of SEQ ID NO: 5
residues 76 to 144 of SEQ ID NO: 5
residues 145 to 210 of SEQ ID NO: 5
residues 34 to 80 of SEQ ID NO: 7
residues 81 to 140 of SEQ ID NO: 7
residues 33 to 79 of SEQ ID NO: 8
residues 80 to 119 of SEQ ID NO: 8
residues 120 to 161 of SEQ ID NO: 8
residues 32 to 91 of SEQ ID NO: 21
residues 25 to 84 of SEQ ID NO: 22
residues 29 to 94 of SEQ ID NO: 24
residues 31 to 85 of SEQ ID NO: 25
residues 1 to 23 of SEQ ID NO: 26
residues 1 to 17 of SEQ ID NO: 27
residues 1 to 28 of SEQ ID NO: 28;

(ii) a homologue of (i);

(iii) a polypeptide containing a relative cysteine spacing of C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C wherein X is any amino acid residue, and C is cysteine;

(iv) a polypeptide containing a relative cysteine and tyrosine/phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z wherein X is any amino acid residue, and C is cysteine, and Z is tyrosine or phenylalanine;

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- (v) a polypeptide containing a relative cysteine spacing of C-3X-C-(10-12)X-C-3X-C wherein X is any amino acid residue, and C is cysteine;
- (vi) a polypeptide with substantially the same spacing of positively charged residues relative to the spacing of cysteine residues as (i); and
- 5 (vii) a fragment of the polypeptide of any one of (i) to (vi) which has substantially the same antimicrobial activity as (i).

2. A protein containing at least one polypeptide fragment according to claim 1, wherein said polypeptide fragment has a sequence selected from within a sequence comprising SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5

10 3. A protein having a sequence selected from SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5.

~~4. An isolated or synthetic DNA encoding a polypeptide fragment according to claim 1.~~

5. The DNA according to claim 4, wherein said DNA has a sequence selected from SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO: 6.

15 6. A DNA construct which includes a DNA according to claim 4 operatively linked to elements for the expression of said encoded protein.

SUB A3
~~7. A transgenic plant harbouring a DNA construct according to claim 6.~~

~~8. The transgenic plant according to claim 7, wherein said plant is a monocotyledonous plant or a dicotyledonous plant.~~

20 9. The transgenic plant according to claim 7, wherein said plant is selected from maize, banana, peanut, field peas, sunflower, tomato, canola, tobacco, wheat, barley, oats, potato, soybeans, cotton, carnations, roses, or sorghum.

10. Reproductive material of a transgenic plant according to claim 7.

25 11. A composition comprising an antimicrobial protein according to claim 1 together with an agriculturally-acceptable carrier diluent or excipient.

12. A composition comprising an antimicrobial protein according to claim 1 together with a pharmaceutically-acceptable carrier diluent or excipient.

13. A method of controlling microbial infestation of a plant, the method comprising:

- i) treating said plant with an antimicrobial protein according to claim 1 or a composition according to claim 11; or
- 30 ii) introducing a DNA construct according to claim 6 into said plant.

14. A method of controlling microbial infestation of a mammalian animal, the method comprising treating the animal with an antimicrobial protein according to claim 1 or a composition according to claim 12.

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15. The method of claim 14, wherein said mammalian animal is a human.
16. A method of preparing an antimicrobial protein, which method comprises the steps of:
 - a) obtaining or designing an amino acid sequence which forms a helix-turn-helix structure;
 - b) replacing individual residues to achieve substantially the same distribution of positively charged residues and cysteine residues as in one or more of the amino acid sequences shown in Figure 4;
 - c) synthesising a protein comprising said amino acid sequence chemically or by recombinant DNA techniques in liquid culture; and
 - d) if necessary, forming disulphide linkages between said cysteine residues.